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Acute Toxicity of Butyl Benzyl Phthalate to the Saltwater Fish English Sole, 'Parophrys vetulus'

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16. ABSTRACT

The Clean Water Act of 1971 directed the Environmental Protection Agency to establish ambient water quality criteria for several classes of elements and compounds, including phthalate esters. Multispecies acute toxicity data are required to develop these criteria. Static bioassay LC_{50}s for butylbenzyl phthalate (BBP) for a single species of marine fish based on nominal doses range from 3 mg/L to 440 mg/L. Flow-through bioassays were used in this study of BBP with shiner perch (*Cymatogaster aggregata*). Using measured exposure concentrations, 96-hr LC_{50}s averaged 0.51 mg/L. Effects on schooling behavior were found at 0.08 mg/L and coloration at 0.24 mg/L. Coupled with the behavioral changes, reduced brain levels of epinephrine found in surviving fish indicated that the mode of acute toxicity for BBP may be through its effects on the catecholamines of the central adrenergic nervous system.

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Acute Toxicity of Butyl Benzyl Phthalate to the Saltwater Fish English Sole, *Parophrys vetulus*

Robert C. Randall,* Robert J. Ozretich, and Bruce L. Boese

U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Marine Division, Marine Science Center, Newport, Oregon 97365

■ Butyl benzyl phthalate (BBP) is a commerically important phthalate ester identified as a priority pollutant by the Environmental Protection Agency. The Clean Water Act of 1977 (PL 95-217) requires the Administrator of the EPA to publish criteria for water quality that accurately reflect current scientific knowledge of the extent and effects of pollutants present in water bodies. Section 304(a)(1) of the Act provides for the nonregulatory, scientific assessment of ecological effects. This work was conducted to provide data for Section 304 on the toxicity of BBP to saltwater fish. The 96-h LC_{50} values found for English sole, *Parophrys vetulus*, were 0.66 (static replenish exposure) and 0.55 mg/L (flow-through exposure) with lethal threshold concentrations of 0.45 and 0.30 mg/L, respectively. Sublethal effects were noted at all BBP exposures, the lowest of which was 0.1 ppm.

Introduction

The EPA has identified phthalate esters both as important industrial chemicals and as priority pollutants (*1*, *2*). Due to their high production volumes (*3*) and widespread use in the plastics industry, they are detected in water (*4–8*), soil and sediment (*5*, *8–10*), fish (*5*, *8*, *10–13*), animal and human tissue (*14–17*), air (*18*), vegetation and minerals (*18*, *19*), and remote areas (*20*). Environmentally, they have relatively slow photolysis, oxidation, and hydrolysis rates, but sorption to suspended solids and sediment, bioaccumulation, and biotransformation/biodegradation are important fate processes (*21*).

Butyl benzyl phthalate (BBP) is one of the important phthalate esters. Its 2.9 mg/L water solubility is relavtively low, with a high octanol/water partition coefficient (log P = 4.8–5.8) (*21*, *22*), suggesting that it would be lipophilic, tend to partition into aquatic life, and adsorb on particulates high in organic content. For this reason, the sorption mechanisms of BBP are anticipated to be relatively important.

Guidelines for deriving water quality criteria are published in the Federal Register (*23*). The minimum data base needed to produce a criterion for saltwater aquatic life consists of acute toxicity levels on at least eight different families, provided that at least two different fish familes are included. Acute toxicity data for BBP exist for one marine fish, *Cyprinodon variegatus*, one invertebrate, *Mysidopsis bahia*, and two alga species (*2*). Reported toxicity values range from 0.9 to 445 mg/L.

LC_{50} values for BBP have been reported far in excess of its solubility (2.9 mg/L), for example, 43 mg/L (*24*) for the bluegill, *Lepomis macrochirus*. Since most available toxicity data for BBP are based on unmeasured concentrations (nominal doses), it is not surprising that investigators have reported unrealistically high concentrations at which lethality occurred. For this reason, at least part of the exposures reported here are documented with measured concentrations. Both static (renewed) and flow-through toxicity tests of the phthalate ester butyl benzyl phthalate (BBP) were conducted with juvenile English sole (*Parophrys vetulus*).

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Table I. Summary of Experimental Data Given as Means with Standard Deviations (in Parentheses)

test	dissolved oxygen, mg/L	pH	temp, °C	salinity, ‰	mean fish weight, g	mean fish length, cm	fish/ tank	fish/L, g
static	7.04 (0.98)	7.3 (0.16)	11.9 (0.5)	24.9 (0.2)	10.7 (2.8)	9.56 (0.68)	10	0.81 (0.22)
flow through	6.61 (0.85)	7.3 (0.14)	12.3 (0.2)	31.4 (0.5)	3.90 (1.8)	6.47 (0.99)	20	3.90 (1.8)

Materials and Methods

Young of the year English sole, *Parophrys vetulus*, were collected by beach seine from the Yaquina Bay Estuary at Newport, OR. They were transported within a few minutes, in an aerated container, to holding tanks with aerated, flowing seawater (25–32‰). After 1 week of quarantine observation, the fish were treated with 0.25% formalin solution for 1 h to remove *Gyrodactylus* parasites and placed into 270-L glass aquaria with aerated seawater flow rates of at least three turnovers per day. Pior to testing, the fish were maintained on an artificial diet, for a minimum of 3 months, composed of 25% squid, 22.5% spinach, 25% beef liver, 2% cod liver oil, 25% wheat germ, and 0.5% Nopstress vitamin mix available from Diamond Shamrock, Fresno, CA.

Except for the absence of bottom substrate, the toxicity tests were conducted in accordance with procedures outlined in American Society for Testing and Materials (25). Butyl benzyl phthalate (product code B-27750) was obtained from Pfaltz & Bauer, Inc., Stamford, CT.

Static (renewed) tests were conducted in 132-L, gel-coated, fiberglass tanks at 12 ± 1 °C with a controlled lighting cycle (16:8 L:D). Five dosage levels plus control were run simultaneously. Ten fish were added to each of the six test tanks for acclimation 1 day prior to the start of the test. Fish were not fed during the test. Eighty percent of the test solution in each test chamber was renewed daily. Water circulation was obtained in each tank by the use of a small submersible pump that was oriented to take water from the surface of each chamber and discharge it at the bottom. The BBP was dissolved in 95% EtOH and added directly to the tank, via the circulation pump intake, to obtain nominal concentrations of 1.5, 1.0, 0.6, 0.216, and 0.130 mg/L. Alcohol concentrations in the test solutions and solvent control never exceeded 0.5 mL/L.

Flow-through tests were conducted in glass, 20-L chambers with 10 volume exchanges per day. Gravity flow of the dilution water from a head tank was controlled by a screw pinchcock to obtain the desired 140 mL/min flow rates. A solution of BBP in 95% EtOH was pumped into each of six dilution water streams at a flow rate of 0.05 mL/min to obtain nominal exposure concentrations of 1.0, 0.60, 0.36, 0.216, 0.130, and 0.00 ppm. The BBP streams were pumped by a Technicon AutoAnalyzer pump equipped with solvent flexible tubing. The bioassay temperature was maintained at 12 ± 1 °C. Alcohol concentrations never exceeded 0.5 mL/L. At the start of the test, 20 fish were added to the already flowing and equlibrated chambers. Fish were not fed during the test.

The seawater used in all tests was obtained from Yaquina Bay, Newport, OR, UV sterilized, and sand filtered prior to use. This water supply has historically been nontoxic in oyster and mussel bioassay and culture (26–28).

Toxicant exposure levels were measured daily in all systems. Toxicant concentrations were determined by a Hewlett-Packard 5880A gas chromatograph equipped with a 15-M fused silica column and flame ionization detector.

The mean analytical precision of the BBP method was 98 ± 3% (n = 10) on standards in the concentration range 0.1–2.6 ppm. The mean recovery of BBP standard additions to filtered seawater and seawater from bioassays was 98 ± 7.1% (n = 32) within a concentration range of 0.1–2.6 ppm.

Fish behavior was observed frequently during the daylight hours and dead fish were promptly removed. Estimations of LC_{50} values (concentrations causing 50% mortality in a specific time period) were calculated by a probit computer program by Finney (29). Wastewater was passed through carbon filters to remove toxicant prior to discharge, and the used carbon was trucked to an approved disposal site.

Results and Discussion

Preliminary exeriments showed that loss of BBP in static tests was significant; 12 h after dosing, toxicant concentrations were 73 ± 6% of the starting concentrations, and after 24 h toxicant concentrations were further reduced to 48 ± 11% of the starting value. Therefore, 80% of all exposure and control water was replenished each day during the tests, and the LC_{50} value is based on nominal concentrations.

We observed a very rapid sublethal effect of BBP on exposed English sole. Within 1 h, fish exposed to toxicant concentrations of 1.0 and 0.6 ppm had significantly slower movements with some loss of equilibrium. After 24 h, fish at all exposure levels were less active than the controls. As exposure time increased, more severe loss of equilibrium occurred. Mouths gaped, and mobility loss was complete near death.

The results of Timofievskaya's (30) work on the effect of phthalate esters, including BBP, on mice, rats, guinea pigs, and rabbits may provide insight to the toxicological mechanism responsible for effects noted on English sole. Clinical poisoning by phthalates was characterized by marked decreases in the summation of subliminal impulses with paralysis evident in poisoned test animals. The most serious phthalogical effect was with central and peripheral nervous system disruptions. Severe paralysis was noted in our English sole test populations that were BBP exposed and intoxicated.

A no-effect level of BBP to English sole was indeterminable since sublethal effects were seen at 0.1 ppm (lowest dose). No acute mortality was noted that static or flow-through BBP exposures of 0.3 ppm or less within 96 h; however, 80% of the test population died at this concentration (0.3 ppm) after a flow-through exposure of 192 h. The lethal threshold concentrations for English sole exposed to BBP were 0.45 (static) and 0.30 ppm (flow through) as determined by graphical extrapolation of the lethality data for 50% and 80% of the test animals (Figure 1).

The nominal static exposure 96-h LC_{50} value for BBP was 0.66 (95% CI 0.53–0.84) mg/L. The measured flow-through exposure LC_{50} value was 0.55 (95% CI 0.48–0.64) mg/L. Water quality parameters listed in Table I were stable and within ASTM guidelines (26).

Other investigators (4, 5, 24) have reported the toxicity of BBP in fish species to be orders of magnitude lower (3–445 ppm) than our data. This difference could be accounted for by species sensitivity. However, since the solubility of BBP is 2.9 mg/L and since most of the pre-

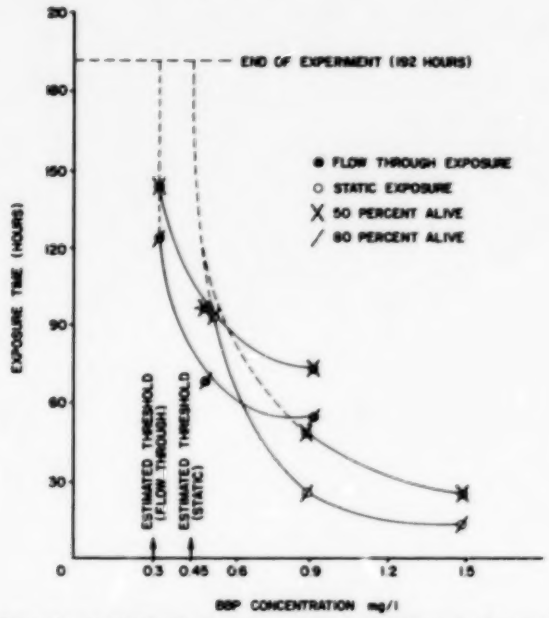

Figure 1. Lethal threshold concentration for English sole, *Parophyrs vetulus*, to (BBP).

vious work has been static, nominal exposure tests, we suspect that the toxicant concentrations actually experienced by those fish might have been orders of magnitude less than reported.

Our results indicate that BBP is acutely lethal to English sole at toxicant concentrations well within its water solubility.

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